Evaluation of Conventional Resistance to European Corn Borer (Lepidoptera: Crambidae) and Western Corn Rootworm (Coleoptera: Chrysomelidae) in Experimental Maize Lines Developed from a Backcross Breeding Program

CRAIG A. ABEL, MARK A. BERHOW,[1] RICHARD L. WILSON,[2] BRADLEY F. BINDER,[3] AND BRUCE E. HIBBARD[4]

USDA–ARS Southern Insect Management Research Unit, P.O. Box 346, Stoneville, MS 38776

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ABSTRACT Plant resistance is a promising control method for the two most damaging insect pests of maize, *Zea mays* L.: the European corn borer, *Ostrinia nubilalis* (Hübner), and the western corn rootworm, *Diabrotica virgifera virgifera* LeConte. Fifteen experimental lines of maize, derived from a backcross breeding program designed to introgress resistance to European corn borer from Peruvian maize into two U.S. Corn Belt adapted inbred lines, were evaluated for resistance to European corn borer and western corn rootworm. The experimental lines were in the second generation of backcrossing. All experimental lines were resistant to leaf blade feeding by European corn borer. These lines had low levels of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one, a chemical commonly associated with leaf blade feeding resistance, indicating that this was not the mechanism of resistance to leaf blade feeding in these lines. Eleven experimental lines were resistant to leaf sheath and collar feeding by European corn borer. Useful sources of European corn borer ovipositional nonpreference and root feeding resistance to western corn rootworm were not identified. Some of the lines evaluated in this study may provide useful sources of resistance to both leaf blade and leaf sheath and collar feeding by European corn borer.

KEY WORDS *Ostrinia nubilalis, Diabrotica virgifera virgifera, Zea mays,* 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one, Peruvian maize, plant resistance

THE EUROPEAN CORN borer, *Ostrinia nubilalis* (Hübner), and the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, cost maize, *Zea mays* L., growers in the United States over 2 billion dollars annually (Metcalf 1986, Mason et al. 1996). Maize resistance to European corn borer is a desirable control method. Conventional maize resistance to leaf blade feeding by European corn borer, based on hydroxamic acid 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) (Klun et al. 1967), has been identified and incorporated into commercially available hybrids (Barry and Darrah 1991). However, because resistance to leaf sheath and collar feeding by European corn borer was more difficult to identify and incorporate into breeding populations, the demand for identifying new sources of resistance remained high. This demand provided impetus for the development of transgenic maize resistant to European corn borer. Maize hybrids that were genetically altered to express the crystal protein (*cry*) genes from *Bacillus thuringiensis* (Berliner) have been successfully developed and marketed (Ostlie et al. 1997). This germplasm provides exceptional European corn borer control; however, insect biotypes may develop that are resistant to the Cry1Ab δ-endotoxin expressed in these transgenic plants (Gould et al. 1997, Tabashnik 1997) and genetically modified maize is not accepted for human use in several countries.

Cultural and chemical control of the western corn rootworm is becoming more difficult because of the insect's tolerance to corn-soybean crop rotation (Levine and Gray 1996), insecticide resistance (Chio et al. 1978, Meinke et al. 1998), microbial degradation of insecticides (Felsot 1989), concerns about soil insecticide toxicity to growers and livestock (Metcalf 1980), ground and surface water contamination (Williams et al. 1988), and poisonings of wildlife and other nontarget organisms (National Research Council 1989). These difficulties have increased the need for research on other control methods, including plant resistance. However, maize expressing resistance to western corn rootworm has been difficult to identify and incorpo-

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[1] National Center For Agricultural Utilization Research, USDA-ARS, 1815 North University Street, Peoria, IL 61604–3999.

[2] North Central Regional Plant Introduction Station, USDA-ARS, Agronomy Department, Iowa State University, Ames, IA 50011.

[3] Corn Insect and Crop Genetics Research Unit, USDA-ARS, Genetics Laboratory, Iowa State University, Ames, IA 50011.

[4] Plant Genetics Research Unit, USDA-ARS, 109 Curtis Hall, University of Missouri, Columbia, MO 65211–7020.

rate into breeding populations (Ortman et al. 1974, Assabgui et al. 1993). Highly heritable sources of maize resistance to western corn rootworm root feeding are greatly needed and would be very beneficial to maize breeders developing resistant varieties.

Non-DIMBOA sources of conventional maize resistance to European corn borer have been identified (Sullivan et al. 1974, Scriber et al. 1975, Chiang and Hudon 1976). Tropical maize populations that have resistance to lepidopterous borers have been identified and have been incorporated into breeding populations (Davis et al. 1988, Smith et al. 1989). Eleven accessions of Peruvian maize were identified as resistant to leaf blade feeding. This resistance is not associated with high DIMBOA levels (Abel et al. 1995). The primary mechanism of resistance was characterized as antibiosis, with larval growth and development times being reduced when larvae were fed maize whorls (Abel and Wilson 1999). Wilson et al. (1995) evaluated these accessions for multiple pest resistance and identified seven accessions resistant to leaf sheath and collar feeding by European corn borer, and two accessions intermediately resistant to western corn rootworm.

The 11 Peruvian maize accessions were used in a backcross breeding program designed to introgress the European corn borer resistant trait(s) into two U.S. Corn Belt adapted inbred lines. The two inbred lines, B94 (Russell 1991) and B97 (Hallauer et al. 1994), were used as separate recurrent parents in the breeding program. Donor plants were selected based on their resistance to European corn borer feeding on leaf blades and on leaf sheaths and collars. Only selected resistant plants were carried forward in the breeding program. Experimental lines have been developed in the second generation of backcrossing (BC2) that seem to resist leaf blade and leaf sheath and collar feeding by European corn borer. However, before this study, these lines had not been tested in a replicated trial for resistance to European corn borer.

For this study, 15 apparently superior European corn borer resistant lines from the backcrossing program that represented each of the 11 original donor parents and the two recurrent parents were selected for evaluation of resistance to root feeding by western corn rootworm, and leaf blade feeding, leaf sheath and collar feeding, and ovipositional nonpreference by European corn borer.

Materials and Methods

European Corn Borer Leaf Blade Feeding. *Field Evaluation.* Fifteen experimental lines, a resistant inbred line check (CI31A), a susceptible inbred line check (WF9), and the two recurrent inbred line parents used in the backcross breeding program (B94 and B97), were grown in a randomized block design replicated four times at the USDA-ARS North Central Regional Plant Introduction Station (NCRPIS), Ames, IA, in 1997. Standard maize production procedures for Central Iowa were used. The maize accessions were planted in single rows on 13 May 1997. Twenty-five seeds were planted per row. Rows were 6.0 m long and spaced 1.5 m apart.

Test plants were artificially infested with insects at the V4–V6 stage of maize development (Benson and Reetz 1985). Approximately 250 neonate European corn borers (provided by the USDA-ARS Corn Insects and Crop Genetics Research Unit, Ames, IA) were deposited into the whorl of the first six plants in each row by using an applicator developed by Mihm (1983). Three weeks after infestation, the test plots were visually rated for European corn borer leaf blade-feeding damage using a 9-class rating scale developed by Guthrie et al. (1960). With this scale, resistant accessions receive the lowest numeric ratings. Plot mean values were used for analysis of variance (ANOVA). Data were analyzed using the ANOVA-2 program of MSTAT-C (MSTAT Development Team 1989). When F values for treatments were significant at the $P = 0.05$ level, means were separated with the RANGE program of MSTAT-C using the least significant difference (LSD) test ($\mu = 0.05$).

Laboratory Analysis of DIMBOA, MBOA, DIM_2BOA, and HMBOA. The remaining uninfested whorls from the European corn borer leaf feeding field evaluation study conducted at Ames, IA, were harvested at the V4–V6 stage, placed in paper bags, and frozen within 30 min at −20°C. The frozen whorls were removed from refrigeration and lyophilized (Labconco model 75050, Labconco, Kansas City, MO), milled to a fine powder using a Wiley mill (Arthur H. Thomas, Philadelphia, PA) with a 0.5-mm sieve, and stored at −20°C in 500-ml glass jars until needed for analysis. Samples were not replicated.

One gram of dried whorl tissue from each entry was extracted at ambient temperature overnight using 10–15 ml of distilled water and a mechanical shaker. The extract was acidified to pH 3 using 1.0 M HCl and stirred for 1 h. Solid material was removed by centrifugation (8,000 × RPM's/20 min) and filtration (Whatman No. 1 filter paper, Hillsboro, OR). The supernatant was partitioned (3×) against ethylacetate. The combined ethylacetate fractions for each sample were evaporated, and the residue was resuspended in 1.0 ml of 50:50 methanol:dimethylsulfoxide.

An aliquot of the material was injected into a high-performance liquid chromatography (HPLC) (Licrosphere reverse phase C-18 column [5 micron, 250 × 4.6 mm], Dionex, Sunnyvale, CA) system for analysis. A linear gradient from 10% methanol to 56% in 0.01 M phosphoric acid was developed over 30 min at a flow rate of 1 ml/min with a dual pump system (Shimadzu model 6A, Columbia, MD). Peaks were detected by a photodiode array detector (Hewlett–Packard 1050A, Palo Alto, CA) monitoring at 265 nm, which stored full spectra of all peaks. The retention times for 2,4-dihydroxy-6,7-dimethoxy-1,4-benzoxazin-3-one (DIM_2BOA), 2-hydroxy-7-methoxy-1,4-benzoxazin-3-one (HMBOA), DIMBOA, and 6-methoxybenzoxalinone (MBOA) were ≈21, 23.5, 24.2, and 28 min, respectively. The peaks were identified by comparison of retention time and spectra with those published by Xie et al. (1991) and by comparison with

standards (Sigma, St. Louis, MO). Relative peak values were calculated to mg/g of dry weight. The rank of resistance ratings and relative levels of DIMBOA, MBOA, DIM_2BOA, and HMBOA were correlated with one another in all possible combinations using Spearman's coefficient of rank correlation (r_s) (Steel and Torrie 1980).

European Corn Borer Leaf Sheath- and Collar-Feeding Evaluation. The 15 experimental lines, a resistant inbred line check (CI31A), a susceptible inbred line check (WF9), and the two recurrent inbred line parents used in the backcross breeding program (B94 and B97), were grown in a randomized block design with four replications at the USDA-ARS North Central Regional Plant Introduction Station (NCRPIS), Ames, IA, in 1997. Standard maize production procedures for Central Iowa were practiced. The entries were planted 13 May 1997. Rows were 4.6 m long and spaced 0.9 m apart. Sixteen seeds were planted per row and each row was thinned to 10 plants.

At anthesis (VT–R1), three leaf axils each above and below the primary ear and the leaf axil at the primary ear were infested with ≈50 European corn borer neonates (provided by the USDA-ARS Corn Insects and Crop Genetics Research Unit, Ames, IA). Eight weeks after infestation, plants were excised at ground level, stalks split lengthwise, and the tunnel lengths caused by European corn borer were measured. Data were analyzed with the ANOVA-2 program of MSTAT-C (MSTAT Development Team 1989). When the F value for treatments was significant at $P = 0.05$, means were separated with the LSD test ($\mu = 0.05$) included in the RANGE program of MSTAT-C. The rank of leaf blade feeding resistance ratings and centimeters of leaf sheath and collar damage for the 15 experimental lines were correlated using Spearman's coefficient of rank correlation (r_s) (Steel and Torrie 1980).

European Corn Borer Oviposition. *Insects.* European corn borers (provided by the USDA-ARS Corn Insects and Crop Genetics Research Unit, Ames, IA) were reared using the methods outlined by Reed et al. (1972) with modifications for the adult stage as described below. Adults emerged in cages (58.7 by 58.7 by 62.7 cm high) made of angle-and-strap aluminum frame (1.9 and 2.3 cm, respectively) and covered on the sides and bottom with 2-mm^2 mesh brass cloth. Cages were constructed so that the brass wire cloth, which inhibits oviposition, covered the inside surface of the cages except for a cloth sleeve in the front. The tops of the cages were covered with 5-mm^2 galvanized wire cloth. This permitted oviposition to occur on wax paper at the top of the cage through mesh screen. Two feeding stations were included in each cage. One feeder was a moist cotton pad suspended from a brass rod 19.5 cm from the top of the cage. The other feeder, a molded plastic unit (10.3 cm^2) with 16 wells (1 ml), was located on the bottom of the cage, and its wells were filled with 1.4% (wt:vol) agar gel containing 39.4% (wt:vol) sucrose (Leahy and Andow 1994). Adult feeding was unrestricted. Three-day-old adult European corn borers, which were at peak oviposition as described by Binder and Robbins (1996), were used for all field oviposition tests.

Field Oviposition Tests. The 15 experimental maize lines, a leaf blade-feeding resistant inbred line check (CI31A), a leaf blade-feeding susceptible inbred line check (WF9), and the two recurrent inbred line parents used in the backcross breeding program (B94 and B97), were grown in a randomized complete block design with four replications at the USDA-ARS North Central Regional Plant Introduction Station (NCRPIS) in Ames, IA, in 1997. Because of insufficient seed for some of the experimental lines, only 10 experimental maize lines and the above listed recurrent parents and checks were evaluated in 1998. Standard maize production procedures for Central Iowa were practiced. For the whorl stage (V6–V8) ovipositional nonpreference test, entries were planted 13 May 1997 in two rows for each replicate using a hand planter, at a rate of four to five seeds per hill, with each row containing 10–11 hills. Rows were 5.2 m long and spaced 0.5 m apart.

For the anthesis stage test (VT–R1), maize accessions were planted (13 May 1997 and 22 May 1998) in three rows for each replicate, at a rate of four to five seeds per hill, with each row containing seven hills. Rows were 4.9 m long and spaced 1.0 m apart. For the whorl and anthesis stage tests, four 2-mm^2 mesh fiberglass screen cages (1.5 by 1.5 by 6.1 m high) and for the anthesis stage test, four 2-mm^2 fiberglass screen cages (2.4 by 2.4 by 6.1 m high) (Synthetic Industries, Atlanta, GA), respectively, were erected immediately over the planted seeds to protect them from external insect attack. On 1 July 1997 and 8 July 1998 all weeds were removed from the cages.

In both tests, plants were thinned to one per hill. For the whorl stage test, 50 pairs of mated European corn borer moths were released into each cage at dusk (2030–2100 hours). On 3 July 1997 (two nights after release of adults), the plants were dissected and the number of European corn borer egg masses was determined. A similar procedure was used for the anthesis stage tests. On 29 July 1997 and 3 August 1998, all weeds were removed from the cages. Plants within hills were thinned to one of each experimental line. Fifty mated pairs of European corn borer moths were released into each cage at dusk on 31 July 1997 and 8 August 1998 for replications 1–4 and 9 August 1998 for replications 5–8, respectively. Two nights after release of adults, egg masses were counted on leaf blades, leaf sheaths, and ears that were dissected from stalks. These plant parts were dissected from the stalks so that the number of egg masses could be accurately counted. The mean number of egg masses was analyzed by PROC GLM (SAS Institute 1989).

Western Corn Rootworm Evaluation. The 15 experimental lines, a resistant inbred line check [NGSDCRW(S2)C4], a susceptible hybrid check (B37 × H84), and the two recurrent inbred line parents used in the backcross breeding program (B94 and B97) were evaluated for western corn rootworm damage. The experimental design was a randomized complete block with four replications at each of two lo-

Table 1. Mean ± SE leaf blade damage by European corn borer and DIMBOA, MBOA, DIM_2BOA, and HMBOA levels expressed in mg/g of dry whorl tissue for 15 experimental lines of maize, two recurrent parents (B94 and B97), a resistant inbred line control (CI31A), and a susceptible inbred line control (WF9)

Entry	Pedigree[a]	European corn borer leaf blade-feeding rating (Ames 1997)[b]	DIMBOA, mg/g	MBOA, mg/g	DIM_2BOA, mg/g	HMBOA, mg/g
Mean Peru donor		2.9	0.030	0.109	0.054	0.165
2-B-3	(PI 503720 × B94)//B94	2.0 ± 0.2def	0.057	0.368	0.025	0.308
27-B-5	(PI 503722 × B94)//B94	1.7 ± 0.2ef	0.005	0.236	0.032	0.046
62-15-3	(PI 503727 × B94)//B94	1.5 ± 0.2f	0.101	0.250	0.051	0.259
66-B-15	(PI 503728 × B94)//B94	1.5 ± 0.2f	0.100	0.383	0.067	0.429
81-9-B	(PI 503731 × B94)//B94	2.0 ± 0.2def	0.066	0.274	0.071	0.329
100-R-3	(PI 503806 × B94)//B94	2.0 ± 0.2def	0.136	0.431	0.070	0.451
107-8-7	(PI 503849 × B94)//B94	1.9 ± 0.2ef	0.180	0.554	0.077	0.420
113-3-1	(Ames 10623 × B94)//B94	2.5 ± 0.2de	0.163	0.403	0.062	0.360
B94		4.0 ± 0.2c	0.130	0.435	0.058	0.310
116-B-10	(PI 503720 × B97)//B97	2.0 ± 0.2def	0.038	0.268	0.059	0.313
131-14-6	(PI 503722 × B97)//B97	2.5 ± 0.2de	0.050	0.401	0.069	0.443
134-16-10	(PI 503723 × B97)//B97	2.2 ± 0.2def	0.017	0.240	0.084	0.291
178-7-7	(PI 503764 × B97)//B97	2.7 ± 0.2d	0.039	0.275	0.074	0.298
191-5-4	(PI 503806 × B97)//B97	2.2 ± 0.2def	0.025	0.248	0.065	0.293
199-13-8	(PI 503849 × B97)//B97	2.1 ± 0.2def	0.025	0.223	0.065	0.247
214-16-6	(Ames 10623 × B97)//B97	2.7 ± 0.2d	0.045	0.323	0.063	0.267
B97		5.8 ± 0.2b	0.108	0.561	0.074	0.348
CI31A		1.7 ± 0.2ef	0.688	0.907	0.085	0.742
WF9		8.5 ± 0.2a	0.022	0.118	0.071	0.113

Means followed by the same letter are not significantly different according to the LSD test ($P < 0.05$), $LSD_{\alpha_{0.05}} = 0.80$. Mean from 11 Peruvian maize accessions resistant to European corn borer leaf blade feeding (Abel et al. 1995).

[a] The method of Purdy et al. (1972) was used for writing the pedigrees of the experimental lines. For example, (PI 503720 × B94)//B94 describes the pedigree for experimental line, 2-B-3. (PI 503720 × B94) designates the F_1 cross that was made. The "//" backcross symbol indicates the recurrent parent, B94, was crossed once to the F_1 generation and then to the first-generation backcross.

[b] Guthrie et al. (1960) 1–9 rating scale: 1–3 = resistance; 4–6 = intermediate in resistance; and 7–9 susceptible.

cations. Plants were grown at two central Missouri locations: University of Missouri Agronomy Research Center (ARC) ≈9.6 km east of Columbia, MO, and the University of Missouri Greenley Farm near Novelty, MO. The ARC location was planted 11 May 1998 and the Greenley Farm location on 15 May 1998. Twelve kernels of each entry were hand planted in each 1.5-m plot, and each plot was thinned to eight plants at the V3 stage. Each field was treated with herbicide and fertilizer as commonly practiced in central Missouri. When most of the cultivars had reached the V2 stage of plant development, an agar suspension of western corn rootworm eggs from the USDA-ARS laboratory in Brookings, SD, was injected into the soil. Infestation was done on 28 May 1998 at the ARC location and 1 June 1998 at the Greenley Farm location. An infester modeled after Sutter and Branson (1980) and described by Moellenbeck et al. (1994) was used to deliver western corn rootworm eggs to each side of the corn row (25.4 cm apart) behind two modified anhydrous nitrogen fertilizer knives at ≈10 cm below the soil surface. Eggs were placed in 0.15% USP agar (Perne, Ridgewood, NJ) suspension, and 1,200 viable western corn rootworm eggs were delivered per 30.5 cm of row.

Root rating dates were timed for maximum damage when ≈50% of the rootworm larvae had pupated, or 8 July for the ARC location and 20 July for the Greenley Farm location. All plots were tagged with laminated labels that were stapled to plastic survey tape and tied to the base of one plant per plot before root extraction. After loosening the roots from the soil with a tractor-pulled implement described by Praiswater et al. (1998), four roots from each plot were extracted from the soil and bound together using duct tape. The roots were soaked in tap water, rinsed, and rated for corn rootworm damage according to the Hills and Peters (1971) root damage scale: 1 = no visible rootworm feeding scars, 2 = visible feeding scars, 3 = at least one root chewed to within 3.75 cm of the stalk, 4 = 1 node of roots chewed to within 3.75 cm of the stalk, 5 = 2 nodes destroyed, and 6 = 3 or more nodes destroyed. According to this system, a rating of three is considered economic damage. We modified Hill and Peters scale (1971) to include 2.5, 3.5, 4.5, and 5.5 ratings. A 2.5 had heavy feeding and some pruning, but not to within 3.75 cm of the stalk. A 3.5 had a half a node destroyed, a 4.5 had one and a half nodes destroyed, and a 5.5 had two and a half nodes of roots pruned within 3.75 cm of the stalk. The four root ratings for each plot were averaged, and an ANOVA was conducted using PROC GLM (SAS Institute 1989). All sources of variance (i.e., location, replication within location, and treatment by location) were considered random with the exception of treatments. The correct *F*-test was conducted for treatments using the RANDOM option (McIntosh 1982, SAS Institute 1989).

Results and Discussion

European Corn Borer Leaf Blade Feeding. *Field evaluation.* The entries differed in their resistance to leaf blade feeding by European corn borer ($F = 37.0$;

df = 18, 50; $P < 0.01$). All 15 experimental lines were resistant (i.e., ratings ≤ 3.0) (Table 1). Thirteen of the 15 experimental lines (except 178-7-7 and 214-16-6) were as resistant as the resistant check, CI31A. The 15 experimental lines had significantly lower leaf blade-feeding ratings than the two recurrent parents, B94 and B97. The original donor parents had higher levels of European corn borer leaf blade-feeding resistance (Abel et al. 1995) than the recurrent parents. It is presumable that the gene(s) conferring the resistance in the experimental lines originated from the donor parents used in the backcrossing program. Because the recurrent parents had intermediate levels of leaf blade-feeding resistance, it is also possible that the resistance identified in the BC2 experimental lines is a quantitative trait resulting from additive genes from both parents, or favorable gene combinations could have been made when crossing the donor and recurrent parents.

Laboratory Analysis of DIMBOA, MBOA, DIM_2BOA, and HMBOA. Concentration of the hydroxamic acids, DIMBOA, MBOA, DIM_2BOA, and HMBOA contained in dried whorl tissue are presented in Table 1. A significant ($r_s = 0.80$, $P = < 0.01$, $t = 4.68$, $n = 15$) rank correlation for levels of relative DIMBOA and MBOA in the experimental lines was observed. This result is expected because the degradation product of DIMBOA, which is present in living tissue, is MBOA. Significant rank correlations of DIM_2BOA and HMBOA (both homologs of DIMBOA) were expected, however, only correlations between DIMBOA and HMBOA ($r_s = 0.59$, $P = 0.02$, $t = 2.62$, $n = 15$) and MBOA and HMBOA ($r_s = 0.87$, $P = < 0.01$, $t = 6.36$, $n = 15$) were significant.

Most of the BC2 lines that used B97 as the recurrent parent (except 134-16-10 for DIM_2BOA and 131-14-6 for HMBOA) had similar or lower levels of DIMBOA, MBOA, DIM_2BOA, and HMBOA than B97. This indicated that there was no additive effect of both parents producing resistant maize in these BC2 experimental lines. Some of the BC2 experimental lines using B94 as the recurrent parent had similar or higher levels of DIMBOA, MBOA, DIM_2BOA, or HMBOA as B94. This may indicate that leaf blade-feeding resistance present in these experimental lines is composed of the additive effect of both parents.

Rank correlations were conducted to determine if relative levels of DIMBOA, MBOA, DIM_2BOA, and HMBOA had an effect on leaf blade-feeding ratings for the experimental lines. There was no significant rank correlation for the experimental line leaf blade feeding ratings and relative MBOA, DIM_2BOA, and HMBOA ($r_s = -0.16$, $P = 0.56$, $t = 0.60$, $n = 15$; $r_s = 0.09$, $P = 0.76$, $t = 0.32$, $n = 15$; $r_s = -0.24$, $P = 0.38$, $t = 0.91$, $n = 15$, respectively), and leaf blade feeding ratings and relative DIMBOA had a significant negative correlation ($r_s = -0.52$, $P = 0.05$, $t = 2.18$, $n = 15$), indicating that DIMBOA, MBOA, DIM_2BOA, and HMBOA did not have a major role in reducing leaf blade feeding in the experimental lines.

Abel et al. (1995) determined leaf blade feeding resistance of Peruvian donor parents was not caused by DIMBOA; however, the chemical or morphological basis of resistance has not been identified. Results of Binder et al. (1999) indicate that water-soluble factors from the resistant Peruvian maize donor parents inhibited the growth, developmental time, and survival of European corn borer. Identifying the chemical basis of resistance would improve the effective use of this germplasm for maize improvement by helping determine, where, when, and to what degree the resistance factor is expressed in the plant.

European Corn Borer Leaf Sheath- and Collar-Feeding. The ANOVA for the European corn borer leaf sheath- and collar-feeding test showed highly significant differences among entries ($F = 93.8$; df = 19, 56; $P < 0.01$). Eleven of the experimental lines rated resistant (<15.2 cm of stalk tunneling) and four were rated as intermediate (15.3–30.5 cm of stalk tunneling) in resistance to leaf sheath and collar feeding by European corn borer (Table 2). Experimental lines 81-9-B, 131-14-6, 134-16-10, and 191-5-4 were as resistant as 116-B-10, which had the shortest stalk tunnels among the experimental lines. All of the experimental lines had shorter stalk tunnels than their respective recurrent parents, B94 and B97. This indicates that the gene(s) conferring leaf sheath- and collar-feeding resistance that are present in the experimental lines originated from Peruvian donor parents used in the backcrossing program.

The experimental lines with B97 as their recurrent parent ($n = 7$, mean = 11.2 ± 0.6 cm) had less stalk tunneling compared with the experimental lines with B94 as their recurrent parent ($n = 8$, mean = 16.5 ± 1.3 cm). A two-sample t-test assuming unequal variances was conducted, and the difference in stalk tunneling was significant ($t = 3.69$, df = 9, $P < 0.01$). B97 has intermediate levels of sheath- and collar-feeding resistance, whereas B94 is susceptible to such damage. It seems that some of the genes conferring intermediate resistance to B97 had been selected during backcrossing, resulting in experimental lines containing European corn borer sheath- and collar-feeding resistance genes from both the Peruvian maize donor parents and recurrent parent, B97.

None of the experimental lines tested performed as well as the sheath- and collar-feeding resistant inbred B52. This is a highly inbred line with near immunity to sheath and collar feeding (Russell et al. 1971). Experimental lines used in this study are backcross populations that are still genetically variable. Mean variability of stalk tunneling for all of the experimental line plants tested for second-generation damage were from 0.0 to 41.9 cm. Further development into inbred lines could develop populations with higher levels of resistance to European corn borer sheath and collar feeding comparable to B52.

Most of these experimental lines have resistance to both leaf blade feeding (typically caused by first generation European corn borer) and leaf sheath and collar feeding (typically caused by second-generation European corn borer). In maize germplasm adapted for the U.S. Corn Belt, two independent types of resistance active against the first and second European

Table 2. Mean ± SE length of tunnels caused by larval European corn borers and number of eggs oviposited by adult European corn borers for 15 experimental lines of maize, two recurrent parents (B94 and B97), and resistant and susceptible controls

Entry	Pedigree[a]	ECB sheath- and collar-feeding (cm) (Amex 1997)	Mean no. of European corn borer eggs oviposited (Ames 1998)
2-B-3	(PI 503720 × B94)//B94	21.8 ± 0.4c	7.9 ± 14.7b
27-B-5	(PI 503722 × B94)//B94	14.0 ± 0.4e	12.5 ± 14.7ab
62-15-3	(PI 503727 × B94)//B94	19.1 ± 0.4cd	9.3 ± 14.7b
66-B-15	(PI 503728 × B94)//B94	13.0 ± 0.4efg	—
81-9-B	(PI 503731 × B94)//B94	11.7 ± 0.4efgh	—
100-R-3	(PI 503849 × B94)//B94	19.1 ± 0.4cd	—
107-8-7	(PI 503849 × B94)//B94	14.0 ± 0.4e	12.0 ± 14.7b
113-3-1	(Ames 10623 × B94)//B94	19.6 ± 0.5cd	—
B94		43.4 ± 0.4a	14.2 ± 14.7ab
116-B-10	(PI 503720 × B97)//B97	8.6 ± 0.4h	19.6 ± 14.7a
131-14-6	(PI 503722 × B97)//B97	10.4 ± 0.4gh	11.4 ± 14.7b
134-16-10	(PI 503723 × B97)//B97	10.4 ± 0.4gh	—
178-7-7	(PI 503764 × B97)//B97	13.2 ± 0.4efg	14.6 ± 14.7ab
191-5-4	(PI 503806 × B97)//B97	11.7 ± 0.4efgh	13.0 ± 14.7ab
199-13-8	(PI 503849 × B97)//B97	12.2 ± 0.4efg	13.4 ± 14.7ab
214-16-6	(Ames 10623 × B97)//B97	11.9 ± 0.4efg	15.0 ± 14.7ab
B97		17.3 ± 0.4d	11.2 ± 14.7b
CI31A		36.8 ± 0.4b	7.5 ± 14.7b
WF9		41.1 ± 0.4a	11.5 ± 14.7b
B52		4.8 ± 0.4i	—
LSD $\propto_{0.05}$		1.23	7.56

Means followed by the same letter are not significantly different according to the LSD test ($P < 0.05$). —, Denotes entries that were not tested for ovipositional preference because of limited field cage space.

[a] The method of Purdy et al. (1972) was used for writing the pedigrees of the experimental lines. For example, (PI 503720 × B94)//B94 describes the pedigree for experimental line, 2-B-3. (PI 503720 × B94) designates the F_1 cross that was made. The "//" backcross symbol indicates the recurrent parent, B94, was crossed once to the F_1 generation and then to the first-generation backcross.

corn borer generation are known to exist. Recurrent selection procedures were used to combine both types of natural resistance (Barry and Darrah 1991); however, progress in transferring these quantitatively inherited traits was slow. Consequently, when maize was transformed with *cry* toxins, which confer resistance throughout the life of the plant, this new resistance was readily used and incorporated into breeding populations.

It would be useful to know if the leaf blade feeding and leaf sheath and collar feeding resistance for the experimental lines tested in this study are positively correlated. If so, selection for both traits during a breeding program would be comparatively easy. Rank correlations were used to obtain a preliminary indication of whether genetic control of resistance to leaf blade feeding is independent of that for leaf sheath and collar feeding. There was no significant rank correlation between levels of leaf blade feeding resistance and leaf sheath and collar feeding resistance for the 15 experimental lines tested ($r_s = 0.25, P = 0.40, t = 0.91, n = 15$), indicating that the two traits are probably under separate genetic control. A broader correlation study is suggested to determine whether genetic control of resistance to leaf blade feeding is independent of leaf sheath and collar feeding. A study should also be conducted to determine the genetic control of resistance to leaf blade feeding and leaf sheath and collar feeding by European corn borer so that maize breeders can develop effective breeding designs for variety development.

European Corn Borer Oviposition. There were no significant differences in the mean number of European corn borer oviposition egg masses laid on the selected maize at whorl stage and anthesis in 1997 ($F = 1.32$; df = 19, 57; $P = 0.16$; and $F = 1.41$; df = 19, 57; $P = 0.14$, respectively). For 1998 at anthesis, there was a significant difference among entries ($F = 1.87$; df = 7, 13; $P = 0.04$). However, the only significant difference was that there were fewer egg masses on entry 107-8-7 than on 116-B-10 (Table 2). None of the experimental lines were different from their recurrent parent, B94. Only 116-B-10 was different from its recurrent parent in the B97 group, having significantly more eggs oviposited. Marston and Dibble (1930) and Beard (1943) attributed increases in European corn borer oviposition to increased plant height and delayed maturity. Experimental line 116-B-10 flowers 2–7 d later and is 4–16 cm taller than the other experimental lines evaluated. Height and maturity differences may account for the increased number of eggs oviposited on 116-B-10. From this study, we conclude that none of the experimental lines are a significant source of ovipositional nonpreference to European corn borer.

Western Corn Rootworm Evaluation. For the western corn rootworm test, there were no significant differences among entries ($F = 1.77$; df = 19, 19; $P = 0.11$). Location differences were significant ($F = 31.1$; df = 1, 65; $P < 0.01$), however, there was no significant difference for the treatment by location interaction ($F = 1.15$; df = 19, 109; $P = 0.31$), indicating that separate analysis for each location was not needed.

In conclusion, the experimental lines evaluated were resistant to leaf blade feeding and leaf sheath and collar feeding by European corn borer. It is possible

that not all of the resistance genes present in the donor parents were introgressed into any one experimental line. To develop maize breeding lines with higher levels of resistance, these experimental lines could be recombined and recurrent selection could be applied under feeding pressures from European corn borer. If recombination of the experimental lines was done, it would be important to combine only those experimental lines that used the same recurrent parent because the two recurrent parents are from different heterotic groups of maize (Russell 1991, Hallauer et al. 1994). Recombining maize from two different heterotic groups would reduce the degree of heterosis possible when producing hybrids (Lonnquist 1974).

The 15 BC2 lines were all developed under selection pressure from the European corn borer alone. Resistance to other insect pests was identified in the donor parents (Wilson et al. 1995) and is still present in some of the BC2 experimental lines evaluated in this research (Abel et al. 2000). Chemical or morphological factors conferring resistance to European corn borer observed in this maize have not been identified. The basis for resistance may have broad insecticidal properties affecting multiple insect species. It is also possible that the basis of resistance is different, but the genes conferring it to the multiple insects are closely linked. An understanding of the genetic control of these resistance traits would allow an effective breeding program to be designed for incorporating these traits into high yielding cultivars.

Experimental line 116-B-10 is highly resistant to European corn borer leaf blade feeding and leaf sheath and collar feeding (Tables 1 and 2) as well as being resistant to leaf blade feeding by fall armyworm, *Spodoptera frugiperda* (J. E. Smith), and leaf blade and stalk feeding by southwestern corn borer, *Diatraea grandiosella* Dyar (Abel et al. 2000). This line may be a useful source of resistance to several lepidopterous maize pests. Like the other experimental lines, it is genetically variable. If an inbred line is developed, future selection for resistance during the inbreeding process could develop progeny with higher levels of resistance to all of the insects tested in this study.

Maize hybrids genetically altered to express the (*cry*) genes from *Bacillus thuringiensis* have been successfully developed (Ostlie et al. 1997). With the potential development of maize insect biotypes resistant to transgenic plants (Gould et al. 1997, Tabashnik 1997), the resistant experimental maize lines evaluated in this study may be needed to delay the onset of such resistance. Also, these experimental lines may offer unique resistance factors to help combat the crop's lepidopterous pests. Further research is needed to investigate the efficacy of stacking or pyramiding these new resistance factors with *B. thuringiensis* maize to increase the durability of the transgenic resistance.

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